

## ABSTRACT OF THE INVENTION

Analytical reagents and mass spectrometry-based methods using these reagents for the rapid, and quantitative analysis of proteins or protein function in mixtures of proteins. The methods employ affinity labeled protein reactive reagents having three portions: an affinity label (A) covalently linked to a protein reactive group (PRG) through a linker group (L). The linker may be differentially isotopically labeled, e.g., by substitution of one or more atoms in the linker with a stable isotope thereof. These reagents allow for the selective isolation of peptide fragments or the products of reaction with a given protein (e.g., products of enzymatic reaction) from complex mixtures. The isolated peptide fragments or reaction products are characteristic of the presence of a protein or the presence of a protein function in those mixtures. Isolated peptides or reaction products are characterized by mass spectrometric (MS) techniques. The reagents also provide for differential isotopic labeling of the isolated peptides or reaction products which facilitates quantitative determination by mass spectrometry of the relative amounts of proteins in different samples. The methods of this invention can be used for qualitative and quantitative analysis of global protein expression profiles in cells and tissues, to screen for and identify proteins whose expression level in cells, tissue or biological fluids is affected by a stimulus or by a change in condition or state of the cell, tissue or organism from which the sample originated.